

Original Research Paper

# Occurrence and Seasonal Variation of Antibiotics in Fez-Morocco Surface Water

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**Abstract:** The presence and accumulation of antibiotics in the water environment has become emerging contaminants of concern causing disruption of ecosystems worldwide. We describe here the seasonal variation and the occurrence of antibiotic residues in Fez city surface water (Morocco). During one year between February 2014 and January 2015, 8 surface water samples were collected monthly. Quantification of the 7 antibiotics was performed by on-line Solid Phase Extraction (SPE) liquid chromatography–tandem mass spectrometry (LC–MS/MS). A total of 96 surface water samples were investigated and the results revealed that 100% of the sites were contaminated by at least one antibiotic. Amoxicillin had the highest concentration with maximum concentration (4107 ng L<sup>-1</sup>), followed by ciprofloxacin (1058 ng L<sup>-1</sup>) and sulfamethoxazole was the most widely detected (93%). Seasonal variation showed that the concentration of antibiotics was higher in winter for trimethoprim (96 ng L<sup>-1</sup>), ciprofloxacin (438 ng L<sup>-1</sup>) and in summer for amoxicillin (1113 ng L<sup>-1</sup>), sulfamethoxazole (162 ng L<sup>-1</sup>) and erythromycin (47 ng L<sup>-1</sup>). The results from this research show that antibiotics are frequent contaminants in Fez city surface water. This is the first attempt to assess the occurrence of these 7 pharmaceutical residues in water samples in Fez Morocco.

**Keywords:** Contamination, Antibiotics, Surface Water, LC/MS/MS, Seasonal Variation, Fez

## Introduction

Water, the essential substance to humans and the source of life, can become a serious hazard to our health, as well as to the life of the flora and fauna. Water bodies are generally sources of drinking water for many people and are used in agriculture for irrigation processes. Water quality can be affected by the increasing discharge of organic contaminant coming from different applications.

The presence of antibiotic in water bodies have been drawing extensive attention because of their high

water solubility, resistance to degradation and potential risks to ecosystem and human health (Tang *et al.*, 2015). These emerging contaminants may originate from different sources and many studies have shown that antibiotics were introduced to the environment from Wastewater Treatment Plants (WWTPs) (Leung *et al.*, 2012; Proia *et al.*, 2016; Zhang *et al.*, 2017), hospitals (Szekeres *et al.*, 2017), or land application of animal manure (Karcı and Balcioglu, 2009). Aquaculture (Cabello, 2006), and industrial garbage (Collado *et al.*, 2014; Shishir *et al.*, 2011).

Several studies from different countries reported contamination by antibiotics in tap water (Cai *et al.*, 2015), surface water (Batt and Aga, 2005), groundwater (Ruixue *et al.*, 2015), sediments (Kim and Carlson, 2007), wastewater treatment plant effluents (Khan *et al.*, 2013). In Morocco, the antibiotics consumption has increased from 9.68 in 2003 to 13.85 Defined Daily Dose/1000 Inhabitant/Day (DDD) in 2012 (Inouss *et al.*, 2015). The public has easy accessibility to antibiotics in low and middle-income countries, from a variety of sources, including drugstores, over-the-counter chemical shops, hospitals, and roadside stalls (Lerbec *et al.*, 2014). Antibiotics can sometimes be bought at pharmacies and drugstores without prescription, despite prohibiting legislation's to stop selling (Bekoe *et al.*, 2014; Lerbec *et al.*, 2014). Extensive accessibility to antibiotics in developing countries could lead to continuous exposure to antibiotics via food and water (Belaïche, 2014). Therefore, the existing treatment facilities of wastewater treatment plants, which are designed for removing biodegradable organics and nutrients, cannot effectively eliminate these recalcitrant chemicals, leading to considerable discharge of pharmaceuticals into aquatic environments (Jin-Lin and Wong, 2013; Tamtam *et al.*, 2008). The use of wastewater for irrigation is common in low and middle-income countries (Drechsel and Keraita, 2014). Usually, untreated and/or partially treated wastewater from urban areas is discharged into drains, smaller streams and other tributaries of larger water bodies, where it is mixed with

storm and freshwater (diluted wastewater) before it is used by farmers. This surface water is referred to as low quality water (Raschid-Sally and Jayakody, 2008).

Thus, the monitoring program of the present study is being implemented for determining the occurrence and seasonal variation of seven antibiotics in Fez city surface water (Morocco).

## Material and Methods

### Target Chemicals

In this study, 7 molecules of antibiotics belonging to several families were selected as target chemicals regarding their rates of consumption in either human or veterinary practices in Fez city (Belaïche, 2014), their detections and persistence in the water environment. Nevertheless, these molecules have been chosen in accord to the availability of analytical standards in the market. Detailed information of the target chemicals is summarized in Table 1.

### Description of Study Area

Oued Fez is the main water body crossing the city of Fez in Morocco (Derwich *et al.*, 2008). It is a tributary of the Sebou River, the biggest river system in Morocco with its 40,000 km<sup>2</sup> catchment (Fig. 1). The river flows in an easterly direction from the springs of "Ras el Ma" (elevation = 420 m a.s.l.) through the Fez medina and into the Sebou, 4 km downstream from the city of Fez (elevation = 210 m a.s.l.).

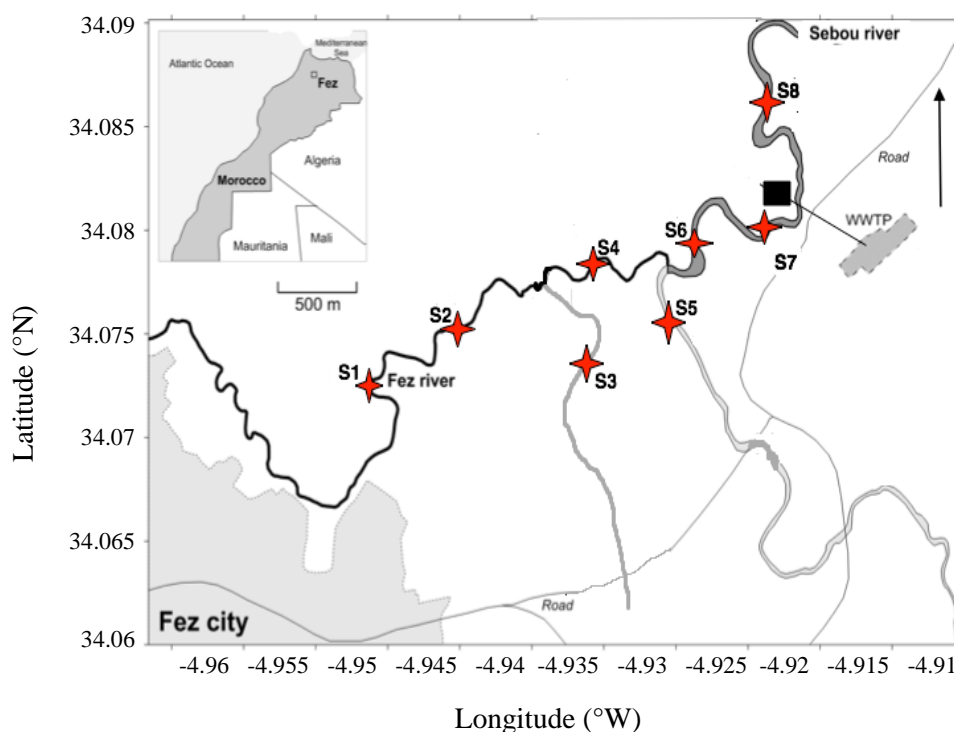


Fig. 1: Sample sites locations on Fez water course

**Table 1:** Uses of selected antibiotic targets

Family	Compound	Uses
β-lactams	Amoxicillin	Veterinary, Human
Tetracycline	Tetracycline	Veterinary, Human and Pisciculture
Macrolide	Erythromycin	Human
Sulfamide	Sulfamethoxazole	Veterinary, Human
Fluoroquinolone	Ciprofloxacin	Human
Quinolone	Oxolinic Acid	Veterinary
Diaminopyrimidines	Trimethoprim	Veterinary, Human and Pisciculture

**Table 2:** Geophysical coordinates, land use and location of the sampling points

Sampling points	Geophysical Coordinates	Land use	Sections of the main stream
S1	34°2'25.03''-5°3'40.21''	Rural with farming activities	Upstream
S2	34°3'19.14''-5°0'14.62''	Urban	
S3	34°2'31.35''-4°59'38.63''	Urban	Midstream
S4	34°3'19.66''-4°58'29.85''	Urban with farming activities	
S5	34°3'13.49''-4°58'59.95''	Urban	
S6	34°4'28.71''-4°57'36.14''	Urban	
S7	34°4'29.77''-4°56'20.36''	Rural with farming activities	
S8	34°4'48.61''-4°54'58.31''	Rural with farming activities	Downstream

Its main course is 33 km long and its catchment area is 615 km<sup>2</sup> (Lombard-Latune *et al.*, 2010). The climate is characterized by hot and dry summers and cold winters. Diurnal temperature swings are large; the mean range is 17-34°C in July and 4-15°C in January. The temperature can, however, rise above 40°C in the summer, especially when the desert wind, is blowing from the Sahara, and fall below 0°C in winter. The rainfall maximum is in the winter period whereas the summer period is almost completely dry and daily hours of bright sunshine varies between about 6h in December and 11 H in July (Perrin *et al.*, 2014). All of Fez's sewage estimated 38 million m<sup>3</sup> (or 110.000 m<sup>3</sup>/j) by Autonomous distribution control of water and electricity Fez) is flushed directly into nearby watercourses. This includes industrial effluents generated by many industries including tanneries, oil mills, metal works, potteries, resulting in serious degradation of water quality. Tanneries, where the different processes are still carried out traditionally, are probably the most polluting and flush, day after day, considerable amounts of chemicals including chromium and ammonium in addition to organic matter into the river (Nazer *et al.*, 2006; Prabhavathy, 2010).

### Sampling Schedule

During one year between February 2014 and January 2015, we collected 96 surface water samples from various urban environments (S1-S8) to cover major land use types in Fez city (Fig. 1), which includes rural areas with farming activities (n = 36), urban (n = 48) and urban area with farming activities (n = 12) (Table 2).

Water samples were collected from the surface of water bodies with 1L glass bottles, which has been conditioned before sampling to avoid contamination by organic compounds. Sample bottles were stored in a cooler with ice until transportation to the laboratory. The

samples were filtered using 0.7 μm Whatman glass fiber filters immediately after collection. Filtrates were stored at -18°C until analyses.

## Experimental

### Reagents and Chemicals

The following antibiotics were purchased from A2S via CIL-Cluzeau (Sainte-Foy-la-Grande, France): amoxicillin (AMO), erythromycin (ERY), sulfamethoxazole (SMX), tetracycline (TET), ciprofloxacin (CIP), oxolinic acid (OXO). Trimethoprim (TRI) was meanwhile purchased from LGC Standards (Molsheim, France).

<sup>13</sup>C- or deuterium-labelled compounds were used as internal standards (ISs): ethyl-d5-oxolinic acid (OXO-d5) was purchased from Sigma-Aldrich and Amoxicillin 3 H<sub>2</sub>O; phenyl-<sup>13</sup>C<sub>6</sub>,99% (<sup>13</sup>C-AMO), erythromycin, N,N dimethyl -<sup>13</sup>C<sub>2</sub> (<sup>13</sup>C-ERY), sulfamethoxazole, ring-<sup>13</sup>C<sub>6</sub> (<sup>13</sup>C-SMX), ciprofloxacin; HCL -2,3 carboxyl-<sup>13</sup>C<sub>3</sub>; quinoline-<sup>15</sup>N (<sup>13</sup>C-CIP) were purchased from LGC Standards. All ISs had purity higher than 95% (isotopic purity >99%).

LC-grade methanol (MeOH) and acetonitrile (ACN) were purchased from VWR (Fontenay Sous Bois, France). Ultrapure water (UP-water) was dispensed from an Elga Purelab Maxima water purification system (Elga LabWater, Le Plessis Robinson, France). Analytical grade formic acid (99%), ortho-phosphoric acid (85%), EDTA disodium salt (Na<sub>2</sub>-EDTA) (99%) and NaOH 50% were purchased from Sigma-Aldrich.

For most analytes, commercial stock standard solution of each compound were sold in MeOH or in acetonitrile except for OXO-d<sub>5</sub> and <sup>13</sup>C-AMO who was sold in powder. OXO-d<sub>5</sub> was prepared in MeOH with 0.1% NaOH 50% to increase her solubility. For the same reason, <sup>13</sup>C-AMO was

prepared in UP-water. These stock solutions were stored in amber glass vials at  $-18^{\circ}\text{C}$ .

Working mix solutions of ISs and native compounds were prepared in MeOH ( $0.1\text{ ng }\mu\text{L}^{-1}$ ).

### Sample Collection and Preparation

Fifty milliliter of water samples were filtered through 25 mm glass fiber GF/F filters, nominal cut-off size  $0.7\text{ }\mu\text{m}$  (Whatman, Fontenay Sous Bois, France). pH was adjusted at 7 with 5% ortho-phosphoric acid or 5% NaOH. Then 2 mL of the so-obtained filtrate was passed through  $0.2\text{ }\mu\text{m}$  nylon membrane filters (Millipore, France) and  $0.1\%$  Na<sub>2</sub>-EDTA 1 M and ISs (at  $0.1\text{ ng }\mu\text{L}^{-1}$ ) was added just before analyze. EDTA was used as a chelating agent to reduce antibiotic binding to major cations, thereby promoting analyte retention on the SPE cartridge.

### On-line SPE-LC-MS/MS Analysis of Antibiotics

We used the method On-line SPE-LC-MS/MS as described previously with a few modifications (Dinh *et al.*, 2011). Separation in the LC column was achieved using an Agilent Zorbax Eclipse XDB-C 18 column ( $4.6\text{ mm I.D.} \times 50\text{ mm}$ ,  $1.8\text{ }\mu\text{m}$  particle size) with a  $0.2\text{ }\mu\text{m}$  prefilter upstream to protect the analytical column, the total run time in separation was 15 min and the IS used for quantification were 13C-AMO was used for AMO, 13C-SMX was used for SMX, TRI and TET, 13C-ERY was used for ERY and 13C-CIP was used for CIP and OXO-d5 for OXO.

All steps of the LC-MS/MS analytical methods, the validation of the method and the limits of quantification (LOQs) are detailed in the Supplementary Information.

## Results

### Spatial Distribution of Antibiotic Residues in Surface Water

Results of our study revealed that 100% of the study sites were contaminated by at least one antibiotic (positive detection). The highest positive samples percentage was recorded in downstream respectively with 63% and 59% respectively in S7 and S8, followed by midstream 48% in S3, 46% in S4, 31% in S5 and 57% in S6. Finally, the contamination rate in upstream was lower with 28% and 32% respectively in S1 and S2.

Concerning antibiotic detection, sulfamethoxazole was the most frequent antibiotics detected in all sites (93%) followed by ciprofloxacin (75%). Trimethoprim came in third range with (69%), amoxicillin and erythromycin were detected at (60%). However, the least detected antibiotics were tetracycline and oxolinic acid with 3% (Fig. 2).

Table 3 summarized the variation of antibiotic concentration of the 8 sample sites analyzed during one year from February 2014 to January 2015. The results showed that the maximum concentration was recorded to amoxicillin with maximum of  $4107\text{ ng L}^{-1}$  recorded in site S8 on July and to ciprofloxacin with maximum of  $1058\text{ ng L}^{-1}$  recorded in site S7 on December. Besides, SMX comes in second range with respectively  $553\text{ ng L}^{-1}$  recorded in site S8 on August, followed by ERY with a maximum of  $114\text{ ng L}^{-1}$  recorded in site S6 on December for and TRI with  $264\text{ ng L}^{-1}$  recorded in site S8 on Julyng  $\text{L}^{-1}$ .

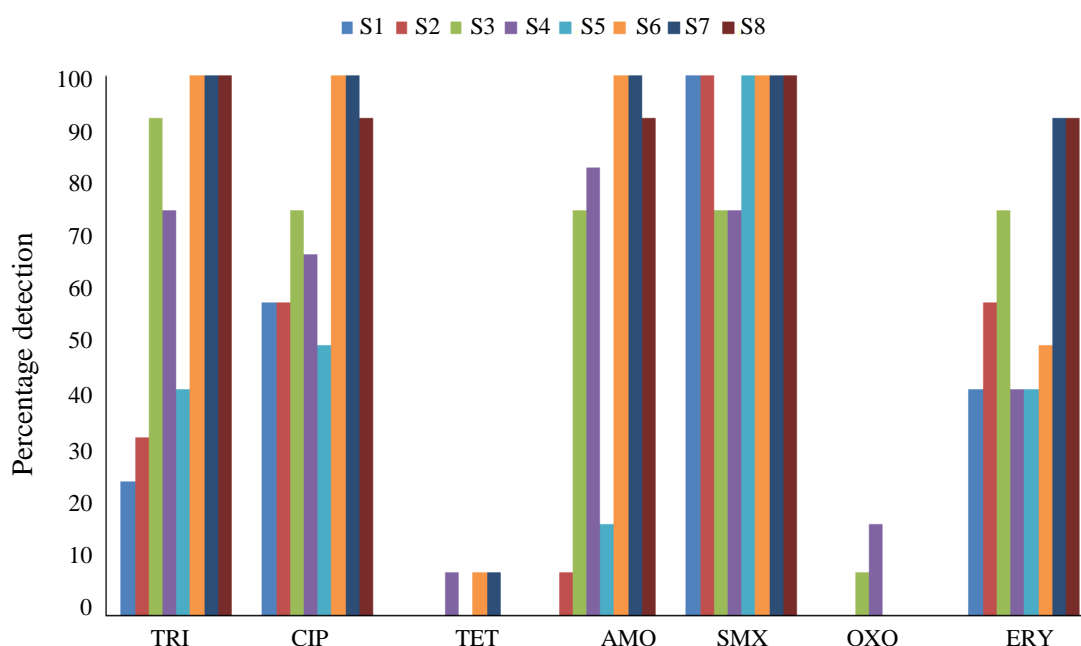
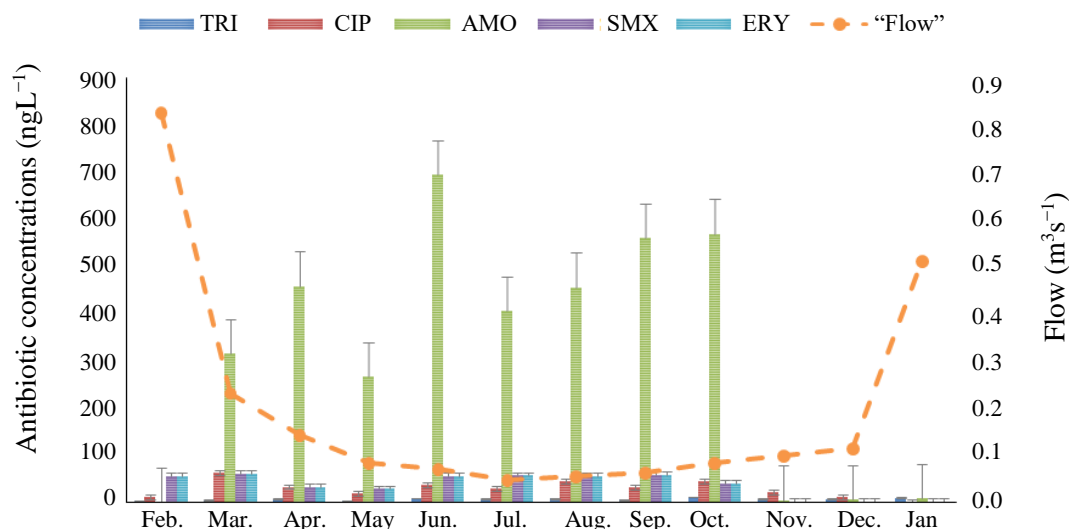


Fig. 2: Detection percentages of antibiotics in 96 surface water samples

**Table 3:** Minimum/maximum, median and extended antibiotic concentrations in ng L<sup>-1</sup> of the 96 samples analyzed

Site		AMO	CIP	SXM	TET	TRI	ERY	OXO
S1	Ranges	<158.3	<7.2-20	2-12	<111.4	<1.9-5	<7.8	<12.2
	Mean	<158.3	7	4	0	1	25	0
	Median	<158.3	8	4	<111.4	<1.9	<7.8	<12.2
S2	Ranges	<158.3-205	<7.2-20	4-47	<111.4	<1.9-5	<7.8-65	<12.2
	Mean	23	62	12	0	1,1	46	0
	Median	<158.3	8	6	<111.4	<1.9	53	<12.2
S3	Ranges	<158.3-2323	<7.2-357	<1.9-327	<111.4	<1.9-44	<7.8-65	<12.2-55
	Mean	914	133	49	0	13,4	38	6
	Median	554	70	17	<111.4	1	55	<12.2
S4	Ranges	<158.3-1350	<7.2-277	<1.9-52	<111.4-286	<1.9-22	<7.8-70	<12.2-64
	Mean	362	34	15	24	6	25	10
	Median	211	9	9	<111.4	7	<7.8	<12.2
S5	Ranges	<158.3-286	<7.2-30	4-35	<111.4	<1.9-7	<7.8-64	<12.2
	Mean	37	6	10	0	2	24	0
	Median	<158.3	6	8	<111.4	<1.9	<7.8	<12.2
S6	Ranges	325-4005	11-1023	12-385	<111.4-134	10-264	<7.8-114	<12.2
	Mean	1165	207	80	11	50	31	0
	Median	708	58	43	<111.4	19	6	<12.2
S7	Ranges	389-3204	26-1058	23-410	<111.4-142	12-242	<7.8-84	<12.2
	Mean	1715	381	182	12	95	45	0
	Median	1687	109	126	<111.4	70	38	<12.2
S8	Ranges	<158.3-4107	<7.2-951	15-553	<111.4	3-120	<7.8-81	<12.2
	Mean	2247	188	178	0	56	30	0
	Median	2247	109	154	<111.4	47	16	<12.2



**Fig. 3:** Annual variations of antibiotic concentrations in midstream

However, the lowest value was recorded to tetracycline with a maximum of 286 ng L<sup>-1</sup> recorded in site S4 on December, followed by oxolinic acid 64 ng L<sup>-1</sup> recorded in site S4 on November (Table 3).

#### Seasonal Variation of Antibiotic Concentrations

Seasonal variation of antibiotic concentration has shown that during periods with high flow, the concentration was 6 times lower than during low flow

periods (Fig. 3). Depending on the season, the river flow ranged from 0.058 to 0.827 m<sup>3</sup> s<sup>-1</sup> which corresponded to a theoretical dilution factor from 6 to 15.

Annual variations of the antibiotic flux in Fez city midstream ranged between from 0.004 to 5.28 g d<sup>-1</sup>, with the presence of two substantial peaks recorded during the winter period (February 2014 and January 2015) respectively, corresponding to high-flow events (Fig. 4).

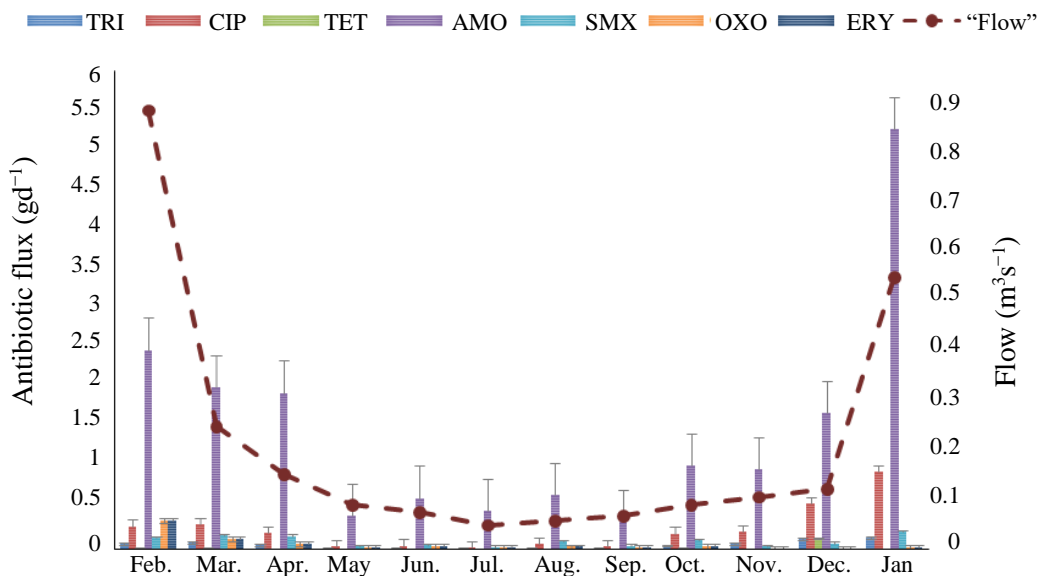


Fig. 4: Antibiotic flux in midstream

## Discussion

Because compound detection and concentration are dependent on source strength, hydrologic condition, timing of sampling and other factors (Focazio *et al.*, 2008) interpretations of these reconnaissance data are limited. Surface water samples recorded higher contamination levels by most of the pharmaceuticals than reported worldwide (Locatelli *et al.*, 2011; Kasprzyk-Hordern *et al.*, 2007; Boix *et al.*, 2015). Once entering into water, these antibiotics may have direct toxicity to aquatic organisms, even at low concentrations ( $\text{ng L}^{-1}$  or  $\mu\text{g L}^{-1}$  level) (Chen and Zhou, 2015). For example, they could cause phytoplankton toxicity, inhibition of microbial activity and change the microbial community structure (Liao *et al.*, 2017). Additionally, antibiotics could promote the development of bacterial resistant (Chaib *et al.*, 2017), which might be potentially harmful to the ecosystem and human health (Zhang *et al.*, 2011).

The level of antibiotics in upstream (sites S1 and S2) were approximately two folds lower than midstream (from sites S3 to S6). This large difference may be directly related to the contamination sources in midstream (urban area), where was little disturbance by human activities and the presence of several private hospitals, clinics, and medical analysis laboratories. In addition, the antibiotic detection percentage was much higher in site S6 (57%) than the other urban sites. In fact, site S6 is a well-known as a hot-spot and has a denser population (around 612489 of inhabitants), this finding highlight the contribution of excessive antibiotics consumption in water environment contamination. Besides, the level of antibiotics in downstream were

much higher than up and midstream. Sites S7 and S8 (rural with farming activities) are impacted by the agricultural activities and the continuously WWTP's discharges into surface water.

We showed that AMO was the most concentrated and SMX was the most frequent in surface water samples.  $\beta$ -lactam antibiotics are among the most frequently used and consumed in large quantities in Morocco and worldwide (Belaïche, 2014), their detection is difficult and the studies which investigated the presence of  $\beta$ -lactam and antibiotics are scarce. because of the poor stability of the  $\beta$ -lactam ring under pH conditions (Moreno-Bond *et al.*, 2009). Some studies were not successful in detecting  $\beta$ -lactam and cephalosporin antibiotics in water matrices (Gros *et al.*, 2013; Zhou and Chen, 2013). Also, SMX is among the most widely used prescribed antibiotics, the large scale of livestock and poultry farming and the high population density result in high usage of SMX (Binh *et al.*, 2018). Levels of CIP found was higher, this result was not surprising because CIP is typically used in human and veterinary medicine to treat and prevent diarrhoea and other intestinal infections (Chen *et al.*, 2012; Liang *et al.*, 2013). Additionally, ERY is widely used in concentrated animal feeding operations to treat bacterial infection diseases of animal and human, such as respiratory diseases, intestinal infections (Lien *et al.*, 2016). More even, TET constitute one of the most extensively used antibiotic classes due to their low cost, ease of use and relatively minor side effects (Li *et al.*, 2016; Ahmed *et al.*, 2017). Tetracycline are also the most widely used veterinary drugs and feed additives in aquaculture and livestock

industries. TET is also added at the sub-therapeutic level to animal feed to prevent infection and act as growth promoters (Sarmah *et al.*, 2006). Besides, OXO is widely used in aquaculture to cure and prevent skin infections in fish. The pisciculture production is usually made in the winter because of the low temperature; the non-negligible fish farming activity present around the city could be involved in the release of the measured OXO (Tamtam *et al.*, 2008).

The seasonal variation of antibiotic concentration has shown that during periods with high flow, the antibiotics concentration was 6 times lower than during low flow periods. This difference in sampling periods can be explained by the variation in antibiotics consumption, physicochemical behavior such as photo-degradation and river flow conditions between summer and winter.

The period of high flows in Oued Fez from December to February (with an average precipitation of 32.6 mm) could lead to dilution on the antibiotics concentration in surface water. Studies have reported that the seasonal variation in antibiotics concentration is related to production, consumption, excretion or environmental factors such as solar irradiation, precipitation, and temperature (Conley *et al.*, 2008). Our results have been confirmed by other studies reporting higher concentrations in summer (Jiang *et al.*, 2011; Gracia-Lor *et al.*, 2012). This variation can partially be explained by the fact that more antibiotics are used and discharged into the aqueous environment during summer because they are utilized to treat higher rates of gastrointestinal infection and diarrhea among both humans and domestic animals (Gracia-Lor *et al.*, 2012). In contrast, levels of antibiotics in waterways increase during the dry season because there is much lower flow runoff in rivers, although the consumption of antibiotics is reduced to some extent due to fewer outbreaks of gastrointestinal infection (Jiang *et al.*,

2011). However, the differences observed in Antibiotic flux in midstream might be related to sediment re-suspension by the flood under high river flow events. Moreover, antibiotic persistence in sediment (Hektoen *et al.*, 1995) and their possible desorption towards the aqueous phase have been reported (Smith and Samuelsen, 1996; Simon, 2005).

The worldwide comparison of the 7 pharmaceuticals in the river surface water is presented in Table 4. The highest concentration of each of the pharmaceuticals obtained in the streams surface water was at moderate to high levels of contamination compared to the concentrations in other regions of the world. In the case of  $\beta$ -lactams for example, AMO (4107 ng L<sup>-1</sup>) was detected at much higher levels than that of Brazil (8.9 ng L<sup>-1</sup>, Locatelli *et al.*, 2011), United Kingdom (245 ng L<sup>-1</sup>, Kasprzyk-Hordern *et al.*, 2007). For the macrolide, ERY (114 ng L<sup>-1</sup>) was detected at a higher concentration than that of Spain (5 ng L<sup>-1</sup>, Boix *et al.*, 2015), Serbia (9.1 ng L<sup>-1</sup>, Petrović *et al.*, 2014) but was much lower than that of the China (810 ng L<sup>-1</sup>, Gulkowska *et al.*, 2006). In addition, OXO (64ng L<sup>-1</sup>) was detected at low level than that of France (140 ng L<sup>-1</sup>, Tamtam *et al.*, 2008) and higher than that of Denmark (5ng L<sup>-1</sup>, Sørensen *et al.*, 2004).

## Conclusion

The results of this study confirm that several antibiotics residues originating from domestic, hospital waste and farming contaminate Fez surface water. This finding is very interesting and may constitute the basis for future work on other Moroccan cities surface water and other environmental matrices. There is a need to continuously predict the concentrations of these antibiotic compounds and to design strategies to minimize exposure to these compounds.

**Table 4:** Global comparison of antibiotic concentrations in surface water

	Antibiotics	Our results	Other results	Reference
Surface water	Amoxicillin	159 to 4107ng L <sup>-1</sup>	8.9 ng L <sup>-1</sup> 39 to 245ng L <sup>-1</sup>	Kasprzyk-Hordern <i>et al.</i> (2007) Locatelli <i>et al.</i> (2011)
	Trimethoprim	2 to 264 ng L <sup>-1</sup>	6.9ng L <sup>-1</sup> Not detected	Petrović <i>et al.</i> (2014) Locatelli <i>et al.</i> (2011)
	Erythromycin	78 to 114ng L <sup>-1</sup>	470 to 810ng L <sup>-1</sup> 9.1ng L <sup>-1</sup> 5ng L <sup>-1</sup>	Gulkowska <i>et al.</i> (2006) Petrović <i>et al.</i> (2014) Boix <i>et al.</i> (2015)
	Sulfamethoxazole	1.9 to 553ng L <sup>-1</sup>	106ng L <sup>-1</sup> 0.3 to 56.8ng L <sup>-1</sup>	Locatelli <i>et al.</i> (2011) Yan <i>et al.</i> (2013)
	Tetracyclin	111.4 to 286ng L <sup>-1</sup>	39.3 to 142ng L <sup>-1</sup> 11ng L <sup>-1</sup>	Tamtam <i>et al.</i> (2008) Locatelli <i>et al.</i> (2011)
	Oxolinic acid	12.2 to 64ng L <sup>-1</sup>	2 to 5ng L <sup>-1</sup> 25 to 140 ng L <sup>-1</sup>	Sørensen <i>et al.</i> (2004) Tamtam <i>et al.</i> (2008)
	Ciprofloxacin	8 to 1058ng L <sup>-1</sup>	2.5ng L <sup>-1</sup> 0 to 28.2ng L <sup>-1</sup> 0 to 1250ng L <sup>-1</sup>	Locatelli <i>et al.</i> (2011) Petrović <i>et al.</i> (2014) Verlicchi <i>et al.</i> (2014)

Adoption of effective proceeds in WWTP's must be applied to eliminate antibiotics. A risk assessment of antibiotic residues study will be very helpful to target the environmental impacts, to sensitize population and medical professional about the severity of the antibiotic presence in environment and to seek urgent actions.

## Study Limitation

To perceive the variations of the antibiotics in water body, and significant variations among the sampling sites, a risk assessment of antibiotic concentration will be dealt in our next article. Also, there is a need to demonstrate the contribution of hospitals effluent, WWTP's and agricultural activities in the hydrological system.

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## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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